

**In the Specification****1. Please amend page 6, lines 7-13 as follows:**

Figure 6. Ligation of a tetravalent maleimide cluster with peptides of the sequence Ac-ELDKWAC (SEQ ID NO: 1) or Ac-CELDKWA (SEQ ID NO: 2). (a) phosphate buffer (50 mM, pH 6.5-7.5), room temp. , Yields: 91% for 22; 88% for 23.

Figure 7. Ligation of peptides of the sequence Ac-YTSLIHSLIEESQNQQEKNEQELLELDKWASLWNWFC (SEQ ID NO: 3) with the carbohydrate-centered tetravalent maleimide clusters. (a) 1:1 MeCN-phosphate buffer (pH 7.0), room temp., Yields: 82% for 25; 84% for 26.

**2. Please amend page 18, lines 14-26 as follows:**

To examine the usefulness of the synthetic maleimide clusters for multivalent peptide assembling, we first set to prepare two multiple antigenic peptides, using 2F5's epitope ELDKWA (SEQ ID NO: 4) as the model peptides. 2F5 is one of the few broadly neutralizing antibodies isolated that can neutralize various primary HIV-1 strains. The neutralizing epitope of 2F5 was mapped to be ELDKWA (SEQ ID NO: 4) (Muster et al., 1993) For the coupling, a cysteine residue was introduced into the epitope sequence either at the C-terminus (peptide 1) or the N-terminus (peptide 2) during the solid phase peptide synthesis. As expected, the ligation between peptide 1 and the maleimide scaffold 10 is extremely fast and efficient at neutral pH at ambient temperature (Figure 6). A simple HPLC purification gave the tetravalent peptide 22 in 91% yield. We also found that the ligation reaction is equally efficient between pH 6.5-7.5 in an aqueous buffer, which is particularly useful for different peptides that may behave differently under distinct pH. Similarly, the coupling of 10 with the peptide2 that has the cysteine residue at the N-terminus gave the tetravalent 23 in high yield (Figure 6).

**3. Please amend page 21, lines 6-19 as follows:**

Similar to the peptide ligation with the carbohydrate-based clusters, the peptide ligation of the cholic acid-based clusters was found to be highly efficient. As shown in Figure 10, three

different peptides were chosen and tested for the ligation. These include the HIV inhibitor DP178 (P37C), a T-helper epitope from tetanus toxoid (830-844), and a minimum epitope sequence ELDKWA (SEQ ID NO: 4) for HIV-neutralizing antibody 2F5. In the case of the T-helper sequence, a tetra-peptide spacer GSSS was introduced at the N-terminus to increase the aqueous solubility of the otherwise hydrophobic T-helper epitope. Regardless the length and complexity of the peptides, the peptide ligation to the cholic acid-based maleimide clusters gave the desired multivalent peptide clusters (36a, 36b, 37a, 37b, and 37c) in very high yields (Figure 10).

However, when the bromoacetyl template 35 was used, no ligation product could be obtained for the long peptide P37C. In the case of simple, short peptide ELDKWAC (SEQ ID NO: 1), the yield of the desired ligation product 38 was isolated in only 35% yield under optimal ligation conditions, together with mono-and di-substituted by-products. The results clearly show that the maleimide clusters are superior to other functionalized templates for multivalent peptide assembly.

**4. Please amend page 32, lines 22-26 as follows:**

DP178 containing a cysteine at the C-terminus (P37C), Ac-YTSLIHSLIEESQNQQEKNEQELLELDKWASLWNWFC-NH2 (SEQ ID NO: 3): retention time (tR), 15.8 min; ESI-MS, 1532.84 (M+3H) 3+, 1149.80 (M+4H) 4+, 920.12 (M+5H) s+.

The minimum epitope of 2F5 (P7C), Ac-ELDKWAC-NH2 (SEQ ID NO: 1): retention time (tR), 13.5 min ; ESI-MS, 905.53 (M + H) 1+, 453.42 (M + 2H) 2+.

**5. Please amend page 33, lines 1-15 as follows:**

The T-helper epitope derived from tetanus toxoid (830-844), CGSSSQYIKANSKFIGITEL-NH2 (SEQ ID NO: 5): retention time, 13.88 min; ESI-MS: 1431.68 (M + 2H) 2+, 1073.90 (M + 3H) 3+, 716.42 (M + 4H) 4+.

Tetravalent peptide (22). To a solution of Ac-Glu-Leu-Asp-Lys-Trp-Ala-Cys-NH<sub>2</sub> (SEQ ID NO: 1) (peptidel) (15 mg, 16.6  $\mu$ mol) in phosphate buffer (50 mM, pH 7.0, 2.0 mL) and acetonitrile (2.0 mL) was added dropwise a solution of maleimide 10 (3.8 mg, 2.6  $\mu$ mol) in DMF (100  $\mu$ L). After shaken at room temperature for 1 h, the reaction mixture was lyophilized. The residue was purified by RP-HPLC as described in general methods, giving the tetravalent peptide 22 (12 mg, 91%) as a white powder. ES-MS of 22: 1691.6 (M+3H) <sup>3+</sup>, 1269.2 (M+4H) <sup>4+</sup>, 1015.8 (M+5) <sup>5+</sup>.

Tetravalent peptide (23). To a solution of Ac-Cys-Glu-Leu-Asp-Lys-Trp-Ala-NH<sub>2</sub> (SEQ ID NO: 2) (peptide2) (10 mg, 11  $\mu$ mol) in phosphate buffer (50 mM, pH 7.0, 1.5 mL) and acetonitrile (1.5 mL) was added a solution of maleimide 10 (2.67 mg, 1.83  $\mu$ mol) in DMF (210  $\mu$ L). After 1h at room temperature, the reaction mixture was lyophilized and the residue was purified by preparative HPLC as described in General Methods to afford the tetravalent peptide 23 (8.2 mg, 88%). ES-MS of 23: 1692.0 (M+3H) <sup>3+</sup>, 1270.1 (M+4H) <sup>4+</sup>, 1016.1 (M+5) <sup>5+</sup>.